ELISA Kit Components	Amount	Part No.
Anti-Ovalbumin Microwell Strip Plate	8-well strips (12)	6051
Ovalbumin Positive Control	0.65 ml	6052
Ovalbumin Standard 0.2 ng/ml	0.65 ml	6053B
Ovalbumin Standard 0.5 ng/ml	0.65 ml	6053C
Ovalbumin Standard 1 ng/ml	0.65 ml	6053D
Ovalbumin Standard 2 ng/ml	0.65 ml	6053E
Ovalbumin Standard 4 ng/ml	0.65 ml	6053F
Anti-Ovalbumin HRP Conjugate (100X)	0.15 ml	6054
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-6050

Other ELISA kits available from ADI

Human: BD-1, BD-2, BD-3 **and**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgG1, IgG4, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgM, Leptin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP
Chicken: IgY (IgG), IgM
Rabbit: CRP, IgG
Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Publications

ADI's ovalbumin ELISA kit has been used and cited in the following publications:

Vianello F, 2006, J. Immunol., 176: 2902 – 2914 (Levels of OVA secreted in CM from B16/OVA.MSCV and B16/OVA.CXCL12 cells cultured for 24 h were also measured by ELISA)

Instruction Manual No. M-6050

Chicken Egg Ovalbumin

ELISA Kit Cat. No. 6050

For Quantitative Determination of Ovalbumin in Solution

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

INTENDED USE

The Alpha Diagnostics Int'l Chicken Ovalbumin ELISA Kit is an in vitro immunoassay for the quantitation of ovalbumin from chicken egg white. The assay is also suitable for samples other than egg white, such as extracts of foods, vaccines, or other products or processes with proper control for assay compatibility.

RESEARCH USE OF THE TEST

Ovalbumin (egg albumin) is one of the major allergens in chicken egg white, and is often the cause of hypersensitivity reactions to food. It serves as a model allergen, suitable for studying the relationship between structure and function, because the amino acid sequence and post-translational modifications of the protein are known.

Egg allergies occur in about 0.5 percent of the population and in about 5 percent of children with allergies. Because influenza and yellow fever vaccines are both made in eggs, egg proteins (primarily ovalbumin) are present in the final product. Residual quantities of egg proteins found in the influenza vaccine (i.e., about 0.02-1.0 ug per dose) are sufficient to induce severe but rarely fatal hypersensitivity reactions in children with egg allergies. ADI has developed an ELISA kit for the detection and quantification of egg albumin in food products, and is an important tool for the standardization and characterization of such allergens.

PRINCIPLE OF THE TEST

The Chicken Ovalbumin ELISA kit is based on the binding of chicken ovalbumin in samples to two antibodies, one immobilized on the microwells, and the other conjugated to horseradish peroxidase (HRP). After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of ovalbumin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The concentration of ovalbumin in samples and control is calculated from a standard curve of standards prepared from purified ovalbumin.

STORAGE AND STABILITY

The microwell plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit are mono-specific for ovalbumin, showing no cross-reactivity by immunoelectrophoresis or immunodiffusion with any other chicken egg or serum protein. Pooled sera of the following species showed no reactivity above background in the ELISA assay when tested at 1:100 dilution: rat, human, hamster, goat, bovine (or 10% fetal bovine serum), horse, monkey, dog, chicken, rabbit, and guinea pig.

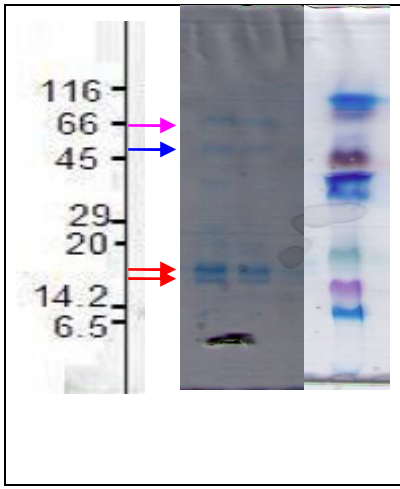
Precision

Samples containing low and high concentrations of ovalbumin were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a 4-parameter curve-fitting program.

Sample	Ovalbumin ng/ml	Intra-assay %CV	Inter-assay %CV
High Value	2.21	**5.6**	**6.0**
Low Value	0.74	**8.6**	**12.9**

Commercial Vaccine Testing

The antibodies used in the ovalbumin ELISA kit have been used to develop a western blot kit as well. Anti-ovalbumin detected ovalbumin in chicken allantoic fluids harvested at 9-11 days.



Allantoic Fluid showed the presence of albumin (66 Kda, ovalbumin, 45 Kda, IgG and other proteins.

Ovalbumin Concn in vaccines by ELISA #6050

Flu Vaccine (Sanofi) = 300 ng/ml
Vaccine made in chicken embryo (allantoic fluid)

MMR Vaccine (Merck) = 0 ng/ml
Vaccine made in chick culture

Allantoic fluid =~340 ng/ml

ADI's ovalbumin ELISA kit detected an elevated concn of ovalbumin protein in vaccines made in chicken embryo.

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, ovalbumin concentrations may be determined as follows:

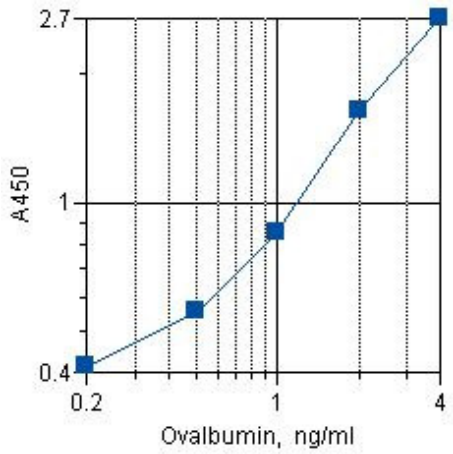
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of ovalbumin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The ovalbumin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 4 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	Concn, ng/ml
A1, A2	**Diluent only Blank**	0.20	
B1, B2	0.2 ng/ml **Standard**	0.41	
C1, C2	0.5 ng/ml **Standard**	0.55	
D1, D2	1 ng/ml **Standard**	0.85	
E1, E2	2 ng/ml **Standard**	1.63	
F1, F2	4 ng/ml **Standard**	2.70	
G1, G2	**Positive Control** [Value: 1.0 – 1.8 ng/ml]	1.31	1.51
H1, H2	**Sample** [Diluted 1:500k]	0.63	0.79
	Calculated: 500k-fold dilution x 0.79 ng/ml = **395** ug/ml in sample		

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely.
Anti- Ovalbumin HRP Conjugate Concentrate (100x) Part No. 6054, 0.15ml	Anti-Ovalbumin-HRP conj. in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Chicken Ovalbumin Microwell Strip Plate	6051	8-well strips (12)	Coated with purified anti-ovalbumin antibodies.
Chicken Ovalbumin Standards			
0.2 ng/ml 0.5 ng/ml 1 ng/ml 2 ng/ml 4 ng/ml	6053B 6053C 6053D 6053E 6053F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing purified ovalbumin; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
Positive Control [Ovalbumin] range on label	6052	0.65 ml	Solution with stated ovalbumin concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter is recommended.; Disposable glass or plastic 5-15ml tubes for diluting samples, and Antibody-HRP Concentrate; Grad. cylinder to dilute Wash Concentrate and Sample Diluent Concn; 200ml to 1L; Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Egg white: Egg white is gelatinous and difficult to pipette; use a wide-mouth pipette to prepare a 1:10 stock solution (e.g., 0.5ml egg white + 4.5 ml of Working Sample Diluent; mix well). The stock can be stored at 2-8^{o}C for a week or frozen in suitable aliquots.

Food, vaccine, other extract: Dilute prepared solutions in Working Sample Diluent at levels that bring the ovalbumin concentration within testing limits. Perform solution-only negative control testing to ensure the compatibility of the sample solution in the assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Antibody-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.
MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Control is provided with the kit, assigned with an Ovalbumin concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Diluent only blank should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Diluent only blank should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

DILUTE Samples in Working Sample Diluent. Dilutions of >1:500K are appropriate for most egg white samples. Dilute other sample types according to expected ovalbumin levels and/or trial testing. DO NOT dilute the Standards or Positive Control.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Before sample addition, add 200-300ul Working Wash Solution to each well and let stand for 5 to 30 minutes.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of Working Anti-ovalbumin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microwell plate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.